NSBRI Immunology, Infection and Hematology Team Strategic Plan

6.0 IMMUNOLOGY, INFECTION & HEMATOLOGY

Team Leader: William T. Shearer, M.D., Ph.D.
Professor of Pediatrics and Microbiology & Immunology
Baylor College of Medicine
6621 Fannin, MC 1-3291
Houston, TX 77030-2399
832-824-1274
832-825-7131 FAX
wshearer@bcm.tmc.edu

6.1 INTRODUCTION

The environmental, inter- and intrapersonal conditions of space flight pose a potential threat to an astronaut's immune system. These conditions include isolation, containment, weightlessness, increased radiation exposure, and enhanced microbial contamination. In all human and animal subjects flown in space, evidence of immune compromise, reactivation of latent virus infection, and development of a pre-malignant or malignant condition exists. Moreover, in all ground-based space flight model investigations, evidence of immune compromise and reactivation of latent virus infection is also observed. Studies are in progress to determine whether malignancy, too, will be observed in these experimental animals. All of these symptoms are similar to those found in a wealth of human pathological conditions where the human immune system is compromised, such as with stress, immunosuppressive drugs, infection, and radiation, and where reactivated, chronic virus infections and cancer appear as a natural consequence. Two examples where these clinical conditions are readily observed are Epstein-Barr virus (EBV)-driven lymphomas in transplanted patients and Kaposi sarcoma in acquired immunodeficiency syndrome (AIDS) patients. Given these known risks to the immune system, it is highly appropriate, indeed imperative, that NSBRI researchers carefully investigate the effects of space flight conditions on human immunity, infection rate, and cancer rate.

6.2 RISKS

The following risks in the Immunology, Infection and Hematology Discipline Area have been identified in the Critical Path Roadmap (risk number in parentheses):

- Immunodeficiency/Infections (22)
- Carcinogenesis Caused by Immune System Changes (23)
- Altered Hemodynamic and Cardiovascular Dynamics Caused by Altered Blood Components (24)
- Altered Wound Healing (25)
- Altered Host-Microbial Interactions (26)
- Allergies and Hypersensitivity Reactions (27)

We have chosen to redefine the risks in a manner better suited to formulation of the present team's research plans. All of these newly stated risks are contained within the original risks.

Risk 1: Radiation Damage to Stem Cell and Immune System
Risk 2: Microgravity and Stress Effects on Immune System and Resistance to Infection
Risk 3: Reactivated Latent Infections
Risk 4: Malignancy
Risk 5: Altered Microbes

In all of the risks proposed for the Immunology, Infection, and Hematology Team, the principal focus must be the underlying stem cell damage that produces the immunological deficits that create the observed risks (e.g., infections on space flights occur because of underlying immune damage).

6.3 GOALS

The Immunology, Infection and Hematology Team has the following goals for its program:

Risk-Based Goals

Goal 1: *Reduce risk of space flight conditions (isolation, containment, stress, microgravity, and radiation) damaging the human bone marrow stem cell and differentiated immune cells.*
Goal 1 covers Risks 1 and 2.

Goal 2: *Reduce risk of astronauts developing new and reactivated infections, premature immune cell death, and malignancy.*
Goal 2 covers Risks 3 and 4.

Goal 3: *Reduce risk of space flight-induced development of superstrains of microbial organisms.*
Goal 3 covers Risk 5.

Non-Risk-Based Goals

Goal 4: *Develop Earth-based applications of space flight studies to help diagnose and treat humans with secondary immunodeficiencies, reactivated viral infections, and malignancy.*

Goal 5: *Integrate research and analysis.*

6.4 DESCRIPTION AND EVALUATION OF CURRENT PROGRAM

The Immunology, Infection, and Hematology Team will seek to reduce the risks defined in Section 6.2 above. The first step in this direction is to firmly establish the molecular and cellular consequences of exposure of the human stem cell and differentiated immune cells (peripheral blood, tissue, mucosa) to the conditions of space flight. Knowing the precise damage to the human immune system will greatly facilitate the development of a countermeasures program. A recent example of the team's progress will illustrate this concept. For 25 years, it has been known that humans in space and in the space-equivalent model of the Antarctic winter display weak, delayed-type hypersensitivity skin reactions to recall antigens. This skin test is a very crude method of assessing immune system deficiencies. Our team has greatly advanced knowledge of the precise molecular events taking place in the human immune system in response to space flight equivalent conditions by determining recently that TH2 $CD4^+$ T-cells reduce the output of the proinflammatory cytokine interleukin-10 (IL-10) in humans in the Antarctic winter. We plan also to define the early cellular changes in reactivated viral infections and the role of the stress (hypothalamic-pituitary-adrenal axis) system in creating secondary immunodeficiency, enhanced infection rates, and development of malignancy. In addition, we will strive to understand the potential for the emergence of superstrains of bacteria, viruses, and fungi in irradiated hosts. With this new information, we will be able to much better plan for an effective countermeasures program involving shielding (structural, chemical) for radiation, stress-reduction programs, nutritional, pharmacologic and immunologic prevention and

treatment programs (for example, gene or cell inhibitors, immunizations and antibody, cytokine, or stem cell therapy), and a microbiocidal program for prevention of opportunistic infection.

Following the award of the present three-year cycle of grant support that began in September 2000, the Immunology, Infection, and Hematology Team was reconstituted with six projects that possessed a cohesive critical mass of investigators and projects (see **Table 6.1**). The principal focus of five of the projects is the harm to the human immune system that might result from immunosuppressive factors in long-term space flight. These factors include deep space radiation, microgravity, physical and psychological stress, isolation and containment, microbial contamination, altered microbial virulence, and sleep deprivation. All of these factors have produced alterations in immune responses of humans and animals flown in space or their counterparts using earth-bound space-equivalent models. There is collaboration of investigators within a project and between projects. The leadership of the team (Drs. William T. Shearer, Janet S. Butel, and Gerald Sonnenfeld), for example, participate in certain aspects of many of the projects (see **Table 6.2**). Projects 1-5 deal with uncovering the pathogenic mechanisms of risk factors, whereas Project 6 concerns the detection system for pathologic microbes, currently bacteria, but in the future viruses and fungi that would cause immunosuppression.

6.4.1 In terms of an overall **team selection of priorities** for a cohesive research program for the **risk-based goals**, we have decided to focus on two types of immunosuppresive factors—radiation and microgravity, using: 1) radiation studies and 2) anti-orthostatic model studies, respectively. All of the six projects will support these two team studies.

6.4.1.1. In the **first of these team projects**, the co-investigators will include Drs. Shearer, Butel, Ling, Conner, Reuben, and Rosenblatt, members of the NSBRI Immunology, Infection and Hematology Team from Baylor and Dr. Daila Gridley from Loma Linda University (LLU). Selected strains of mice (e.g., BALB/c, C57 black) will be exposed to proton and gamma ray radiation and subsequently to murine viruses (e.g., gamma 68, polyomavirus), in an attempt to determine the combined effects of space radiation and latent virus infection on the immune function of study animals. This first approach will examine the simultaneous effects of radiation and infection and will then be followed by a sequential approach of infection first and radiation second, the likely scenario for human space travelers to Mars. The dose of radiation that will be utilized initially (3Gy, the estimate of radiation received by astronauts on a Mars Mission) will be that used by Dr. Gridley and her colleagues who have demonstrated rapid and profound alterations in immune cells and immune responses in murine subjects. Replicate and controlled experiments will be performed by both the LLU site and the Baylor site to insure that the same methods are followed at both sites and that the results of the experiments at Baylor confirm those of LLU. If gamma radiation proves to be equivalent to proton radiation in terms of effects upon the immune system (e.g., spleen cell T-cell response to non-specific stimuli and specific antigen stimulation; plasma antibody formation to neoantigen; spleen lymphocyte subset distribution), it may be possible to avoid transfer of mice between institutions, as Baylor has a source of gamma radiation.

In addition to examination of the effects of radiation and latent virus infection on immune cells and immune responses, study animals will be evaluated for the development of tumors and blood malignancies. This will be carried out with the assistance of Dr. Cory Brayton, a veterinary pathologist at Baylor, who has agreed to collaborate on this project.

Also, Dr. Alan Gewirtz at the University of Pennsylvania has begun collaborative NSBRI studies with Dr. Elizabeth Sutherland at the Brookhaven National Laboratory (BNL) with bone marrow-derived human stem cell lines. These cell lines were exposed to heavy metal ion (Fe^{56}) radiation

and subsequently tested by standard hematologic assays for ability to form colonies of cells in the myeloid series: granulocytes, erythroid cells, and platelets. In the future, similar experiments will be performed at LLU, where the effects of proton and gamma radiation will be evaluated in these same assays. Because the preparation of human stem cells from donor bone marrow also yields precursor cells in the lymphoid system, it will be possible to simultaneously evaluate the effects of the various types of radiation on the development of T- and B-cells. Similarly, macrophages, monocytes, and stromal cells could be evaluated. The methods of analysis of these various types of immune cells could include measurement of cell growth factors (e.g., IL-3, IL-6, IL-7, TGF-β), apoptosis gene regulation (e.g., gene array assay), and cell repair pathways. These studies would include the collaboration of Drs. Gewirtz, Reuben, Rosenblatt, and Gridley.

Also, peripheral blood human stem cells will be harvested by pheresis in subjects given granulocyte-monocyte colony-stimulating factor (GMCSF) to increase the number of circulating stem cells at the M.D. Anderson Cancer Center. Dr. James Reuben will utilize these cell harvests in similar radiation studies and evaluate dendritic cell (#1 and #2 types) function in the presentation of antigens to lymphocytes.

In both bone marrow and peripheral blood stem cell preparations, evidence of genetic damage will be investigated by examination of progenitor cells for chromosomal breaks. These measurements will yield important information on the possibility that radiation of human stem cells might result in leukemogenesis and tumorigenesis.

Future collaborative studies have been proposed for the Radiation Team, in which the use of surrogate markers could be used to assess the risks of tumor development in irradiated animals. Surrogate markers would greatly reduce the time needed to evaluate tumorigenesis and to observe exposed animals for cancer development. For the current Fe^{56} irradiated rat breast tumor model, one such surrogate marker might be the appearance of epithelial cells in the peripheral blood that herald the development of breast cancer. In addition to the detection of epithelial cells, it might be possible to examine the gene imprints of these cells by gene array assays. Such studies might yield a characteristic dysregulation of normal gene activation that would be predictive of breast cancer in this animal model.

This first team project addresses Goals 1 and 2 (space flight conditions damaging immune cells and development of new or reactivated infections, premature immune cell death and malignancy, respectively), but with the assistance of Project 6 we will also be addressing Goal 3, the detection of genetically altered (possibly supervirulent) strains of environment or host microorganisms with the use of DNA probes (see Section 6.5 and Table 6.1). Originally designed to develop genetic probes of spacecraft bacterial contaminants, Project 6 will adapt the genetic probes to detect contaminating viruses and to detect the emergence of both bacteria and viruses made more virulent by exposure to spaceflight conditions, principally radiation.

6.4.1.2 The **second of the team projects** will involve the anti-orthostatic (AOS) (hind-limb suspension) model and addresses Goals 1 and 2. The subgroup on hind-limb suspension felt that it was important that standardized procedures be used by the group to allow for comparison of results across projects. The exact caging and suspension techniques do not have to be identical, but the parameters used for setting up the suspension should be uniform. In the future, all suspension will be set up with uniform parameters. Suspension will be carried out with a 15 to 20 degree head-down tilt. The tilt will be measured at the body axis of the animal. Controls for all experiments will consist of at least vivarium controls in standard housing and restraint controls with animals in suspension hardware but with all four paws on the ground and bearing weight. Additional controls may be added at the investigator's discretion. Vivarium controls

will be in individual cages, not housed with multiple animals per cage. All hind-limb suspension experiments will commence in the morning between 9 and 11 AM. Dr. Sonnenfeld has already had remarkable success with these procedures in demonstrating an at least two-fold increase in death in AOS mice challenged with Klebsiella pneumoniae.

We plan to examine changes in differential gene expression in the immune system using commercially available low-density nylon-based gene array technology. Each blot contains 23 specific and two housekeeping genes. Arrays are available that can detect specific sets of genes that are grouped based on their association with known signal transduction pathways. Once changes in particular pathways are identified, pathway-specific gene arrays are available to elucidate changes in expression of pathway-specific genes. In addition, arrays are available to detect changes in gene expression of mouse cytokines, interleukin receptors, chemokines, chemokine receptors, inflammatory cytokines, T-cell activation markers, and B-cell activation markers. The approach is to catalog global changes in the immune system (cell distributions, cytokine production, gene expression) utilizing the AOS mouse model, and then to determine any additive effects of concomitant virus infection and/or proton irradiation on those patterns. This comprehensive approach will provide new insights into mucosal and systemic host immune functions. Additionally, comparison of the results from the animal model and human studies should provide directions for future studies.

6.4.2 In **planning for the next 5 years of research**, it is expected that the immediate research efforts will be directed toward uncovering the mechanisms behind the changes already observed in immunity due to exposure of subjects to space flight conditions. An example of this process would be the information gained from projects that will examine the effects of protons, gamma rays, and heavy metal ions upon mature lymphocytes in the peripheral blood and the pleuripotent hematopoietic bone marrow stem cells in irradiated mice. By carefully adjusting the timing and dose of irradiation, it will be possible to determine where the lesions due to radiation occur along the primordial stem cell to mature lymphocyte differentiation pathway. There could be multiple hits, indicating that several differentiation steps are affected, or the result could be due primarily to stem cell damage early in the differentiation pathway. Not only would this information be important for understanding the pathogenesis of radiation-induced immunosuppression, but it would also be important for construction of an effective countermeasures program. A lethal hit to the primordial stem cell would mandate the countermeasure of replacement of stem cells, most likely by use of an autologous stem cell transfusion with stem cells harvested prior to space flight and preserved from the same radiation damage. Appropriate repair of immune system damage should restore control of reactivated viruses and microbial infections.

In terms of a 5-10 year plan of research, it is estimated that human evaluation of the countermeasures of the projects will take place in this phase of NSBRI-supported research. It is most likely that the countermeasures will change during the first five years of research, as basic science investigations discover mechanisms unknown at present. Using irradiation of the human bone marrow stem cell development pathway as the example, it will be important to know where radiation effects take place. If the principal radiation damage is to a mature lymphocyte (e.g., $CD4^+$ T-cell), there may not be the need for reconstitution with the primordial stem cell, but rather treatment with cytokines such as IL-7 and IL-12 that produce maturation of early lymphocyte precursors into mature lymphocytes. Thus, the countermeasures proposed for today will yield to the basic scientific discoveries of the first five years of research. Some countermeasures most likely will not change, such as the use of intravenous immunoglobulin (IVIG) in immunocompromised space travelers. IVIG has a half-life of one month and can be used repeatedly in patients until a permanent reconstitution of immunoglobulin by B-cells takes place.

6.4.3 We have initiated plans for the **non-risk goals of our team** as well**.** For Goal 4, Earth-based applications that help diagnose and treat immunodeficiency, reactivation of viral infection and malignancy, we will begin by examining whether during long distance space travel we can utilize the current AIDS model of the $CD4^+$ T-cell count as a predictor of immunosuppression. For this type of prediction system, constant refinement of standards will be very important as illustrated by the fact that the previous cutoff of 500 $CD4^+$ T-cells/μl of blood that defined the limit of beginning immunosuppression has been lowered to 300 cells/μ1. Also, there may be much more suitable surrogate markers that would prove more definitive in alerting space physicians of pending immune compromise. The balance of pro- and anti-inflammatory cytokines (e.g., IFN-γ vs. IL-10), as seen in Antarctic winter dwellers for example, might become a better early warning system. The example given (IFN-γ vs. IL-10 levels in the Antarctic winter model of spaceflight isolation) signaled the simultaneous activation of latent virus shedding. Researchers will now use this cytokine balance to look for early evidence of immunosuppression due to virus infection in children and adults in routine medical practice. Similarly, we will apply whatever we learn for our space-based immunosuppression prediction system for future Earth-based applications.

In further pursuit of Goal 4, the microbial detection system that Dr. Fox is perfecting will enable astronauts to know when water or food sources will give them food poisoning, a condition extremely undesirable in space. It will be important for this microbial detection system to be developed for viruses found in saliva as well so that an early warning system could be activated should latent virus activation occur in astronauts and antiviral therapy need to be initiated. This detection system has many potential Earth-based applications. Another activity towards achieving Goal 4 is to examine whether adaptation of immunoreconstitution therapies to astronauts in space may yield important clues for treatment of humans on Earth, such as the ability to restore a failing immune system before total collapse.

Our team's efforts to achieve the non-risk goal (Goal 5) of integration of research and analysis can be appreciated in **Figure 6.1 and Table 6.2**. At our team retreat in July 2001 and the NSBRI Retreat held in January 2002, we discussed how to interconnect all of the projects by collaborations of investigators and sharing of research specimens and analytical data.

FIGURE 6.1: Integration of NSBRI Immunology, Infection, and Hematology Team

Not only did the team investigators plan for the integration of their own team projects, they also met with investigators of the Radiation Team to plan collaborative projects, particularly the examination of effects of space radiation upon the immune cells, cytokines, and antibodies in animal models that develop malignancies. Also, Dr. Shearer wrote a letter of collaboration to the NSBRI for the new application of Dr. Daila Gridley, LLU, to support her inclusion in the team effort so as to supply the present investigators with a senior radiation biologist/immunologist. Some of the commitments of team collaborators are listed below:

- Drs. Shearer, Butel, Ling, Conner, Reuben, Rosenblatt, and Gridley for studies on radiation, viral infection, and immune responses.
- Drs. Sonnenfeld (also involving Drs. Shearer, Butel, Conner, and Gewirtz as plans develop) and Vazquez (NSBRI Radiation Team) for studies on radiation and immune responses in AOS Mouse Model.
- Drs. Sonnenfeld, Butel, Ling, and Conner for studies of the effects of neuroendocrine hormones on viral growth and replication.
- Drs. Gewirtz, Sutherland, and Reuben for radiation and hematopoietic stem cell research.
- Drs. Sonnenfeld, Fox, and Willson for studies to determine effects of neuroendocrine hormones of gene expression of bacteria by array analysis.
- Drs. Fox, Willson, Butel, and Ling for studies of rapid detection of viruses.
- Drs. Shearer, Rosenblatt, Reuben, Butel, and Ling for Antarctic analog studies. Dr. Desmond Lugg (ANARE) will be a collaborator on these studies, as well.

6.5 OBJECTIVES AND STRATEGIC ACTIVITIES

Presented here are the objectives underlying each goal and the strategy that we plan to use to achieve the goals and objectives of our program.

Goal 1: *Reduce risk of space flight conditions (isolation, containment, stress, microgravity, radiation) damaging the human bone marrow stem cell and differentiated immune cells.*

Goal 2: *Reduce risk of astronauts developing new and reactivated infections, premature immune cell death, and malignancy.*

Since many of the objectives and specific activities for Goals 1 and 2 are similar and interrelated, we have presented them together. They are:

Objective 1A-2A. Assess risk and target level of acceptable risk.

- In human models, develop a system of surrogate markers for the component of the immune system being tested based upon well-established clinical standards (e.g., $CD4^+$ T-cell count and CDC recommendations).
- In animal models, use natural outcomes if the endpoints are reached within days (e.g., death). If the endpoints are to be reached in months (e.g., breast carcinoma), develop surrogate markers of cancer development (e.g., epithelial cells appearing in blood stream).

Objective 1B-2B. Determine mechanisms.

- Complete Antarctic winter-over studies of human pro- and anti-inflammatory cytokine balance in subjects excreting virus.
- Complete study of latent viruses EBV and JCV excretion in Antarctic winter-over human subjects and match data results with those of subjects with altered cytokine balance.

- Match immune and viral study data with psychological profile study of Dr. Joanna Wood on Psychosocial Team.
- Complete study of HPA axis in AOS mice to determine role of catecholamines in lowering immune resistance factors.
- Begin radiation studies of murine model to determine extent of immune compromise and how these animals handle latent virus challenge and whether they develop lymphoreticular malignancy.
- Quantitate immune responses in irradiated and virus-challenged mice and determine effects of radiation on degree of immunosuppression and reactivation of virus.
- Begin in vitro study of proton, gamma, and heavy metal radiation on human pleuripotential bone marrow and peripheral blood stem cells and the myelogenous and lymphocytic differentiation pathways leading to malignancy.
- Determine immune system gene expression profiles in AOS mice before and after virus infection, with and without radiation exposure.
- Begin study of apoptosis in thymocytes of AOS mice to determine effects upon education and selection of lymphocytes.
- Determine TH1 vs. TH2 cytokine profile in AOS mice and effects of immune mediators upon regulation of bone metabolism.
- Increase the specificity of nucleic acid and molecular beacon probes for early detection of microbial contamination of water supply in spaceships.

Objective 1C-2C. Develop countermeasures.

- Form strategic plan for general and specific immunoreconstitution of astronauts based upon the deficits uncovered in exploring the mechanisms of space flight immunodeficiency (see above).
- Based upon current treatments of immunodeficient humans on Earth (i.e., genetically immunodeficient patients, immunosuppressed transplant patients, patients with rheumatoid arthritis, patients with AIDS), plan to adapt therapies involving parenteral immunoglobulin, cytokines, chemokines, monoclonal antibodies, fusion proteins, and autologous stem cells for astronauts who develop secondary states of immunodeficiency in space travel.
- Based upon current and developing treatment regimens for viral infection (e.g., gancyclovir for cytomegalovirus), plan to adapt therapies for astronauts who become infected or re-infected with opportunistic and reactivated viral infections.
- Based upon current and developing experimental treatments of cytokine-mediated (e.g., tumor necrosis factor-alpha) wasting disease, plan to adapt such treatments (e.g., thalidomide) for use in astronauts who develop wasting disease in space due to abnormal TH1 vs. TH2 cytokine balance and apoptosis of lymphocytes.
- Work with Nutrition Team (Dr. Joanne Lupton) to devise nutritional supplements that augment innate and acquired immunity.
- Work with Performance and Psychosocial Teams to provide adequate rest periods for astronauts to restore lymphocyte health.

Goal 3: *Reduce risk of space flight-induced development of superstrains of microbial organisms.*

Objective 3A. Assess risk and target level of acceptable risk.

Objective 3B. Determine mechanisms.

- Isolate and genotype gut flora of irradiated and AOS mice.
- Perform resistance assays of microbes recovered from radiation experiments.

Objective 3C. Develop countermeasures.

- Investigate effects of immunotherapy (e.g. monoclonal antibodies) on drug-resistant microbes.

Goal 4: *Develop Earth-based applications of space flight studies to help diagnose and treat humans with secondary immunodeficiencies, reactivated viral infections, and malignancy.*

Objective 4A. Plan for the future when space flight diagnosis and treatments are being utilized for astronauts with immune compromise, opportunistic infection, and possibly cancer.

- Explore use of T cell count and cytokine balance as indicators of immune compromise.
- Continue efforts to expand microbial detection system to include viruses.

Objective 4B. When such methods of diagnosis and modes of treatment of astronauts in space become a reality, determine whether these methods, dictated by the unique features of space travel (e.g., microgravity, strict regulation of diet, enforced rest periods), might be applied in diagnostic methods and treatment programs on Earth.

Goal 5: *Integrate research and analysis.*

Objective 5A. Integrate research within the Immunology, Infection, and Hematology Team:

- Continue current integration efforts described in **Table 6.2**.

Objective 5B. Integrate research with other teams using modeling, as well as other approaches:

- Enlarge collaboration with Radiation Effects Team. Add Dr. Daila Gridley of LLU to team, if her NSBRI grant application is accepted.
- Continue collaboration with Psychosocial Team (Dr. David Dinges, Dr. Joanna Wood), NASA (Dr. Duane Pierson), and ANARE (Dr. Desmond Lugg) in analyzing: 1) immune factors, 2) virus reactivation, and 3) psychological profiles of Antarctic winter dwellers in a synergy project (submitted to NSBRI for funding [12-13-01]).
- Develop joint projects with Nutrition Team (Dr. Joanne Lupton)
- Develop joint projects with Bone Metabolism Team (Dr. Jay Shapiro)

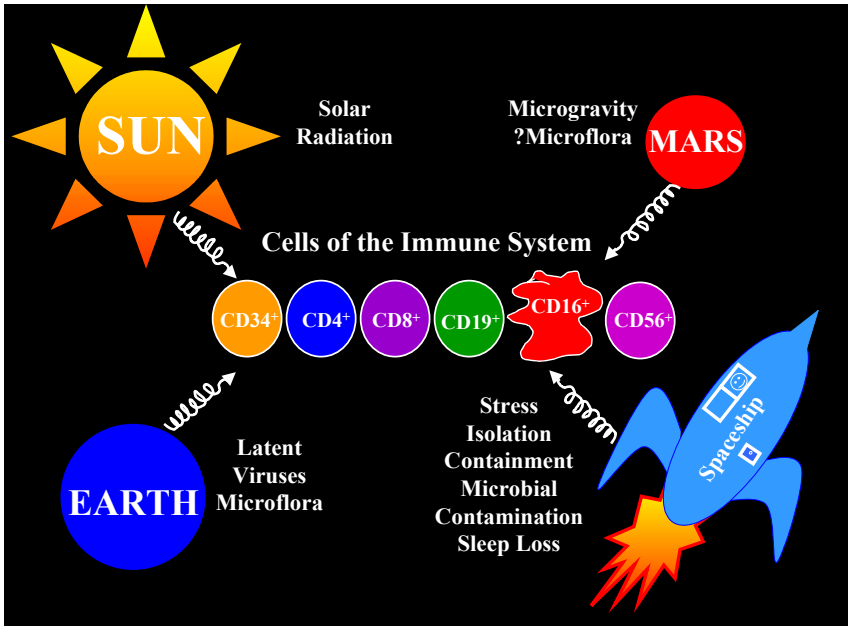
Objective 5C. Integrate research with investigators not formally associated with the NSBRI.

- Continue collaboration of team with Dr. Desmond Lugg (ANARE), Dr. I. Larina (IBMP), Dr. Duane Pierson (NASA), Dr. Marcelo Vazquez (NSBRI radiation team) and Dr. Elizabeth Sutherland (BNL), and Dr. Daila Gridley (LLU).

6.6 SUMMARY

The essence of the challenge facing the investigators of the Immunology, Infection, and Hematology Team is depicted in **Figure 6.2**.

FIGURE 6.2
Conditions of long-term spaceflight that may weaken cells of the immune system.



The voyage to and from the planet Mars is estimated to consume 3 years. During that time, human space travelers will be exposed to stress, microgravity, isolation, containment, sleep disruption, microbial contamination, and solar radiation (up to 3 Gy of proton and gamma radiation). All of these conditions are known or suspected causes of immunosuppression, which is possibly sufficient to lead to reactivation of latent viral infections and malignancy. The immune cells that may be susceptible to these causes of immunosuppression include the bone marrow stem cell ($CD34^+$), helper T-cell ($CD4^+$), cytotoxic T-cell ($CD8^+$), B-cell ($CD19^+$), monocyte-macrophage ($CD16^+$) and natural killer cell ($CD56^+$). Because of the inherent difficulties of assessing these risks in space flight, ground-based models that incorporate some of the conditions of long-term space flight offer the best hope of adequately predicting the harm that may occur to the human immune system in interplanetary travel. Taken from Shearer WT, et al. Antibody responses to phiX-174 in human subjects exposed to the Antarctic winter-over model of spaceflight. *J Allergy Clin Immunol* 2001;107:160-164.

All of the risk factors affecting cells of the immune system by themselves are known to represent risks to earthbound inhabitants. Solar radiation, for example, is known to cause melanomas of the skin, a tumor that is controlled by T-cells. As humans age, T-cell immunosurveillance weakens, and these forms of cancer appear. Unpublished studies by NASA have already shown a 3-fold increased incidence of skin melanomas in astronauts as compared to earthbound NASA employees (295 astronauts compared to 909 controls, Longitudinal Study of Astronaut Health, Surveillance Epidemiology, and End Results Program). Another example is that of reactivated latent viruses, leading to unregulated growth of B-cells and non-Hodgkin lymphomas.

The role of the team is to scientifically define and quantitate the potential harmful effects of the conditions of space travel upon human immune responses and health. Foremost in our efforts will be the constant search for countermeasures to the risks that we define and quantitate. We are not content to merely document the probability that space risk factors exist and are likely to create infections and cancer in astronauts. We want to use the immunological reagents and procedures that are already in use for humans with immunodeficiency on Earth for our astronauts whose immune responses are jeopardized by the immunosuppressive conditions of space travel. We have made progress so far in our team research, and a timeline for the strategic activities for each of our Goals is presented in **Table 6.3**. For Goal 1 **(Table 6.3A)**, the team is already well

into the Countermeasure Development Phase 1 (Focused Mechanistic Research) and beginning to enter Phase 2 research (Preliminary Countermeasure Development Research). We expect to complete Phase 2 research by 2009, but overlapping with development of Phase 3 research (Mature Countermeasure Development Research) by 2006-2010. We anticipate beginning Phase 4 research (Countermeasure Evaluation and Validation) during 2009-2011 and Phase 5 research (Operational Implementation of Countermeasure Strategy) during 2011-2012. Similarly, with Goal 2 **(Table 6.3B)** and Goal 3 **(Table 6.3C)**, we anticipate a steady and progressive development of team research from the present Phase 1 level to Phase 5 level over the next 10 years.

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Table 6.1. Project Research Activities

PI/Project	Risk(s) Addressed	Countermeasure Target	Experimental System	Phase 1 Activities: Focused Mechanistic Research	Phase 2 Activities: Preliminary Countermeasure Development Research	Phase 3 Activities: Mature Countermeasure Development Research
BUTEL/Viral Infections and Mucosal Immunity	1-5	Pharmacological Agents	AOS; IR; Humans	Detect immune damage; Measure infection	Formulate antiviral reagents	
FOX/Microorganisms in the Spacecraft Environment	3-5	Pharmacological Therapy	Microbes	Develop microbe detection system	Perfect microbe detection system	Flight test detection system
GEWIRTZ/Effect of Deep Space Radiation on Human Hematopoietic Stem Cells	1,3,4	Stem Cell Therapy, Cancer Chemotherapy	In Vitro Stem Cells	Detect damage to stem cells	Formulate autologous stem cell transplant	Test stem cell Transplantation in space
SHEARER/Space Flight Immunodeficiency	1,3,4	Antibody Therapy, Stem Cell Therapy	IR; Humans	Measure apoptosis in thymocytes	Adapt Earth Rx strategies	Perform Rx in space
SHI/Endogenous Opioid-Mediated Fas Expression in Stress-Induced Lymphocyte Apotosis	1,2,4	Cytokine Therapy	AOS, IR	Measure HPA in AOS, IR	Formulate drug treatment program	
SONNENFELD/Suspension, the HPA Axis and Resistance to Infection	2-5	Pharmacological Therapy	AOS, IR	Determination of role of different stressors on immune response	Formulate anti-stress program	Test program in space

Risks Key: 1) Radiation damage to stem cell and immune system; 2) Microgravity damage to immune system; 3) Reactivation of latent viral infections; 4) Malignancy; 5) Altered microbes

Definitions: AOS, anti-orthostatically suspended murine model; IR, irradiated mice; Humans, humans exposed to Antarctic winter or isolation in capsules; Microbes, microbial detection systems; HPA, hypothalamic pituitary axis; Rx, treatment

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Table 6.2. Integration Activities

	BUTEL	FOX	GEWIRTZ	SHEARER	SHI	SONNENFELD
Internal Communication (E-mail, telecons, retreats, scientific meetings for all projects)	• Shearer • Sonnenfeld • Fox • Gridley (LLU) • Lugg (ANARE) • Pierson (NASA) • Larina (IBMP)	• Butel • Sonnenfeld	• Shearer • Shi • Sutherland (BNL)	• Butel • Gewirtz • Fox • Shi • Sonnenfeld • Gridley • Lugg • Pierson • Dinges (Psych)	• Sonnenfeld • Butel	• Shearer • Butel • Vazquez (Rad)
Integrated Experiment Development	Model Radiation and AOS Studies • Shearer • Gridley • Reuben • Sonnenfeld • Pierson • Larina • Lugg • Fox	Collaborative Gene Probe Studies • Butel • Sonnenfeld	Model Radiation Studies • Reuben • Shearer	Model Radiation and Human Exposure Studies • Butel • Reuben • Lugg • Gridley	Model AOS Studies • Gewirtz	Collaborative Gene Probe Studies • Butel • Fox
Sample Sharing	Blood, Urine • Shearer • Reuben • Larina	Microbes • Butel • Sonnenfeld	Stem Cells • Reuben • Shearer	Blood • Butel • Reuben • Dinges	Blood • Sonnenfeld • Butel	Blood • Vazquez
Synergistic Studies of Opportunity	Antarctic Winter • Shearer • Lugg • Pierson	Radiation, AOS • Butel • Sonnenfeld	Radiation • Reuben • Shearer • Kennedy (Rad. Team)	Antarctic Winter, Sleep Deprivation • Butel • Dinges • Lugg	Radiation • Sutherland • Kennedy	Radiation, AOS • Butel
Development of Computer Model of Integrated Human Function						

Definitions: ANARE, Australian National Antarctic Research Expedition; NASA, National Aeronautics and Space Administration; IBMP, Institute for Biomedical Problems, Moscow; LLU, Loma Linda University; Psych, Psychosocial Team; Rad, Radiation Effects Team; BNL, Brookhaven National Laboratory; AOS, Antiorthostatic Suspension

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Table 6.3A. Achieving Goal 1: Reduce Risk of Space Flight Conditions (Isolation, Containment, Stress, Microgravity, Radiation) Damaging the Human Bone Marrow Stem Cell and Differentiated Immune Cells

Countermeasure Development Phases	Pre 2001	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Phase 0: Observational & Phenomenological Research													
Phase 1: Focused Mechanistic Research													
• Define radiation damage to immune system in animals													
• Define microgravity damage to immune system in animals													
• Define latent virus damage to immune system in animals													
• Measure virus excretion in Antarctic-bound humans													
• Develop surrogate markers of immunity for space													
Phase 2: Preliminary Countermeasure Development Research													
• Adapt antibody therapy to space travel													
• Adapt cytokine therapy to space travel													
• Develop space immunization strategies													
• Develop radiation shielding methods													
• Plan stem cell therapy in space													
Phase 3: Mature Countermeasure Development Research													
• Perfect and test ready-to-use antibody auto injectors													
• Perfect and test cytokine auto injector system													
• Safety study of space vaccines													
• Safety study of autochthonous (self) stem cell transplants													
Phase 4: Countermeasure Evaluation & Validation													
• Efficacy study of Phase 3 countermeasures (above)													
Phase 5: Operational Implementation of Countermeasure Strategy													
• Spacecraft trial of countermeasures													

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Table 6.3B. Achieving Goal 2: Reduce Risk of Astronauts Developing New and Reactivated Infections, Premature Immune Cell Death, and Malignancy

Countermeasure Development Phases	Pre 2001	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Phase 0: Observational & Phenomenological Research													
Phase 1: Focused Mechanistic Research													
• Measure virus excretion in Antarctic-bound humans													
• Measure tumorigenesis in irradiated/virus-infected animals													
• Study malignant transformation in irradiated human stem cells													
• Study additive effect of malnutrition on tumorigenesis													
• Develop surrogate markers for premalignancy													
Phase 2: Preliminary Countermeasure Development Research													
• Develop radiation shielding methods (chemical, physical)													
• Develop oncogene array assays for gene targeting													
• Adapt cytotoxic T-cell rescue of EBV tumors for space													
• Develop cancer virus vaccine for space													
Phase 3: Mature Countermeasure Development Research													
• Safety study of radiation blockers (animals)													
• Safety study of cancer gene promotor inhibitors (animals)													
• Safety study of anti-EBV tumor T-cells (humans)													
• Safety study of cancer virus vaccine (humans)													
Phase 4: Countermeasure Evaluation & Validation													
• Efficacy studies of Phase 3 countermeasures (above)													
Phase 5: Operational Implementation of Countermeasure Strategy													
• Spacecraft trial of countermeasures													

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Table 6.3C. Achieving Goal 3: Reduce Risk of Space-Flight-Induced Development of Superstrains of Microbial Organisms

Countermeasure Development Phases	Pre 2001	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Phase 0: Observational & Phenomenological Research													
Phase 1: Focused Mechanistic Research													
• Genotype gut flora of irradiated mice • Perform resistance assays of organisms of irradiated mice • Combination of microgravity and radiation to determine microbe virulence assays • Adapt Fox gene probes to viral organisms													
Phase 2: Preliminary Countermeasure Development Research													
• Develop monoclonal antibodies to resident organisms • Develop cytokine therapies to augment cytotoxic T-cells • Develop fusion proteins specific for receptors on cancer cells • Develop toxin-antibody conjugate molecules for cancer													
Phase 3: Mature Countermeasure Development Research													
• Safety study of monoclonal antibodies • Safety study of cytokine activation of T-cells • Safety study of fusion proteins for cancer • Safety study of toxin-antibody conjugates													
Phase 4: Countermeasure Evaluation & Validation													
• Efficacy study of Phase 3 countermeasures (above)													
Phase 5: Operational Implementation of Countermeasure Strategy													
• Spacecraft trial of countermeasures													